

BEHAVIOR OF THE CELL SURFACE DURING CLEAVAGE. VIII. ON THE CLEAVAGE OF MEDUSAN EGGS *

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Coelenterate eggs cleave characteristically with a one-sided advance of the furrow on the animal side, in contrast to utter quiescence on the vegetal side.¹ Around the beginning of the present century various theories were proposed to account for the mechanism of such division but they were highly hypothetical in nature, and failed to acquire much popularity. The present study, although admittedly preliminary in nature, is an attempt to clarify some aspects of the division of medusan eggs.

In previous papers the authors have postulated that the primary cause of cleavage is spindle elongation, both in regularly cleaving eggs such as those of the sea-urchin (Dan, 1943b) and also in cells with excentric nuclei (Dan and Dan, 1947). But in the asymmetrical furrow formation of eggs of the latter type, for example in the heart-shaped cleavage of *Astriclypeus manni*, there are secondary peculiarities not found in regular cleavage. The first is rotation of the asters about their centers, so that their respective animal hemispheres converge. This results from asymmetrical sheath ray formation, and can be detected by noting the change in direction of bending of the rays in successive stages of cleavage (Dan and Dan, 1947). The second peculiarity is the bending of the spindle, so that it becomes convex toward the vegetal pole. This is a direct result of the astral turning. Of course, such bending is possible only because the spindle itself is somewhat rigid, and the connections between the spindle tips and astral centers are firm.

Now, among different batches of *Astriclypeus* eggs, the excentricity of the spindle varies and the degree of one-sidedness of the furrow varies correspondingly. An orderly arrangement of these eggs, from less to more excentric spindles, will form a series continuous with the mode of cleavage of medusan eggs (Fig. 1). This might mean that medusan cleavage simply represents an extreme case of heart-shaped cleavage. If so, astral rotation and spindle bending should also be found in medusan eggs. The following investigation will test these propositions. Moreover, a few points specific to the present material will be presented.

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¹ Opinions differ as to which side of the medusan egg should be considered the animal pole. Ordinarily, the side at which the polar body is extruded and the cleavage furrow begins is called the animal pole. Schleip (1929) and Korschelt (1936), however, have adopted the opposite terminology. This is because the egg axis and the larval axis run in opposite directions among the Ctenophora. In order to avoid confusion, the terms "polar body pole" and "micromere pole" are sometimes used. In this paper, the term "animal pole" denotes the polar body pole and the term "vegetal pole" refers to the micromere pole, thus preserving a unity of expression throughout this series of papers.

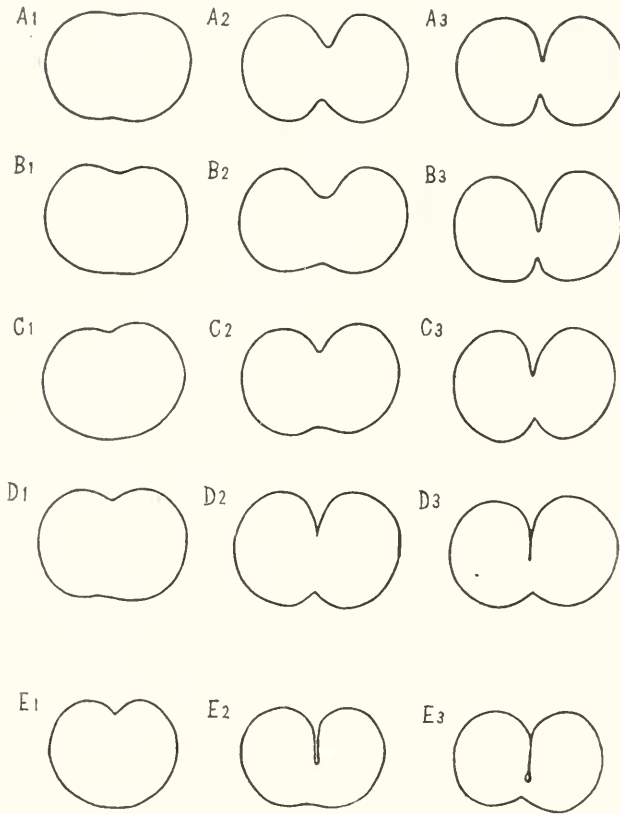
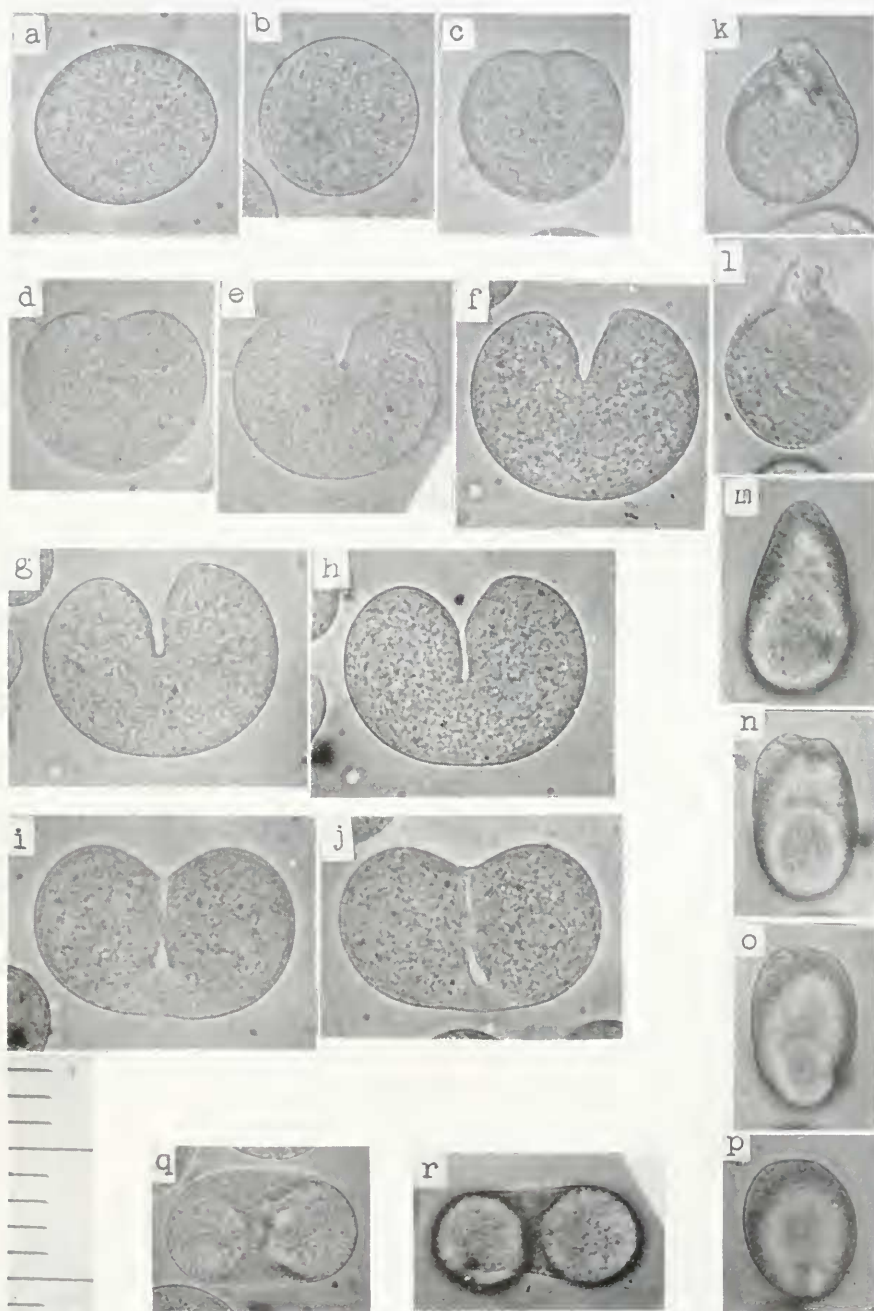


FIGURE 1. Comparison of the cleavage modi of sand-dollar eggs (*Astriclypeus manni*) and medusan eggs (*Spirocodon saltatrix*). A-D, cleavage of sand-dollar eggs arranged in order from less excentricity to more excentricity of the mitotic figure. E, cleavage of the medusan egg. Note that these make a continuous series.

The eggs of *Spirocodon saltatrix*, one of the *Tubularioanthomedusae*, are used throughout this work. This medusa is quite abundant in the bays around the Misaki Station during the winter. The animals spawn on being put in the dark.

PLATE I. (a) Short time after fertilization. The egg pronucleus is vaguely visible at the extreme left side of the cell, which is the animal pole. (b) Conjugating pronuclei and the resulting mitotic figure are invisible by the usual microscopic observation. (c) and (d) Beginning of furrow formation. (e) Slightly more advanced stage. (f)-(j) Successive stages of cleavage of the same egg. (f) The spinning is not yet evident. (By this time, the fine protoplasmic processes must be coming out from the furrow surface; these are not visible unless observed under oil immersion.) (g) The amalgamation of the protoplasmic processes is partly completed. (h) (i) (j) The amalgamation products spread between the blastomeres in a sheet, fastening the cells together. (k)-(p) Views along the spindle axis. (k) (l) Initial stages of cleavage which roughly correspond to stage (c) of the side view. (m) More advanced stage. Note that the still uncleaved part between the blastomeres can be vaguely seen as a bright circle. (n) (o) (p) Successive stages of cleavage of a single egg. Notice that as the connecting bridge narrows down, the blastomeres round up. (q) View from above (animal pole). (r) View from below (vegetal pole). The smallest division of the scale is 10 μ .

PLATE I



(Uchida, 1927). The reaction is particularly clear-cut if the animals have previously been exposed to electric light. Around 10°C. , the first cleavage takes place from $1\frac{1}{2}$ to 2 hours after putting them in the dark, depending upon the latent period for spawning.

These eggs have absolutely no enveloping membrane in either the unfertilized or fertilized condition, as far as can be judged by microscopical observation. The egg pronucleus is clearly visible in the unfertilized condition and vaguely so during a short time after fertilization, taking an extremely excentric position within the egg (Plate Ia). But by the time syngamy approaches, it is lost from view (Plate Ib). When mitosis begins, the asters are discernible, although the rays are very faint and require oil immersion to be definitely distinguished (Plate II). For this reason, sectioning is necessary for detailed observation of the internal structures. When unfertilized eggs of *Spirocodon* are fixed in a chrome-formol mixture, the internal cytoplasm remains fairly homogeneous throughout the cell (Fig. 2; 1). But if eggs are fixed by the same mixture after fertilization, the peripheral cytoplasm assumes a coarsely vacuolar aspect (Fig. 2; 2, 3, 4, 5). On the other hand, if fixed by osmium, the cytoplasm of fertilized eggs is homogeneous (Fig. 3). From the above facts, it can be assumed that the peripheral vacuoles of the chrome-formol-fixed eggs are artifacts. If fertilized eggs are over-stained by neutral red or by Nile blue sulphate, many large heavily stained vacuoles appear in the peripheral zone of the cells. Such over-stained cells have already lost the capacity to cleave.

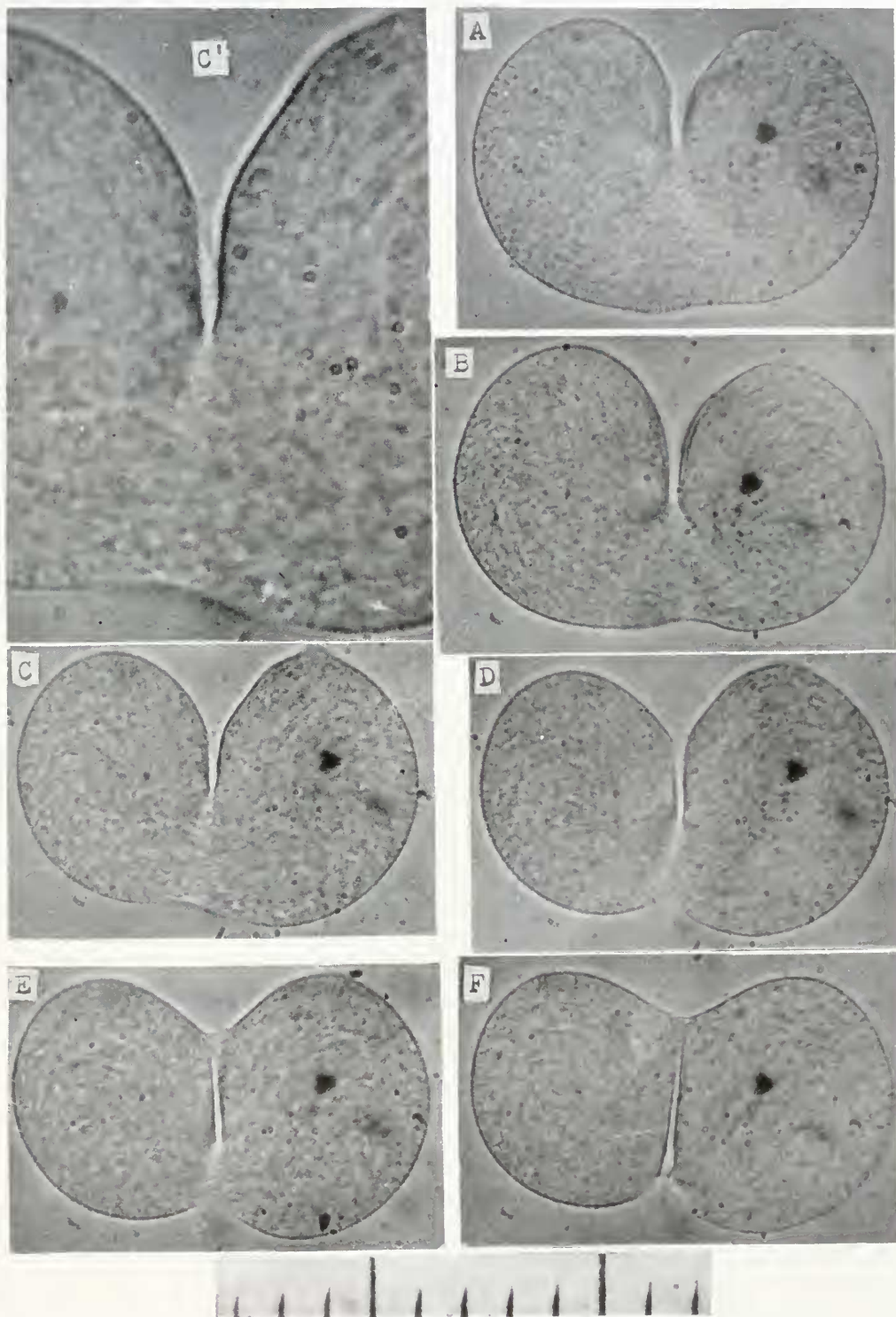
NORMAL CLEAVAGE OF THE EGGS OF SPIROCODON

Since the asters of this egg cannot be seen with certainty unless oil immersion is used, it is rather difficult to anticipate the exact time of the onset of cleavage. Most observations, therefore, were started after a slight indication of furrow formation had been noted. This drawback was somewhat offset by the slowness of the advance of the furrow.

When the furrow deepens, the egg tends to come to lie on its side, with the egg axis parallel to the substratum. As for the changes in contour in the side views of these cells during cleavage, there is not much to add to what has been reported for other forms (Plate I, *b* to *j*). If viewed from other angles however, an interesting feature appears. Photographs of two views perpendicular to the side view and to each other are shown in Plate I, *k* to *r*. From these, it is clear that cleaving *Spirocodon* eggs form flat plates of squarish shape. This is the reason why they tend to come to lie on their sides. Although sea-urchin eggs elongate in the direc-

PLATE II. Successive stages of cleavage of a single *Spirocodon* egg observed with oil immersion lens. (A) Slight indication of the vortex figure within the blastomeres. No sign of the spinning activity within the furrow. (B) The vortex configuration is becoming more evident. The spinning activity has not begun yet. (C) The vortex figure is at its climax. The amalgamation of the protoplasmic processes is proceeding (*see C'*). (C') Enlargement of C showing the spinning activity in the furrow. Note a longish shadow along the furrow space and several very faint lines running across the furrow, nearer the tip of the furrow. The latter are threads formed by the fusion of several protoplasmic processes which cannot be shown in the photograph. The former is the amalgamation product of the latter. This grows by further amalgamation into a sheet. (D) The spinning is complete around the entrance of the furrow. (E) Astral rays (vortex figure) are becoming vaguer. (F) The furrow has almost reached the vegetal side. The smallest division of the scale is 10μ .

PLATE II



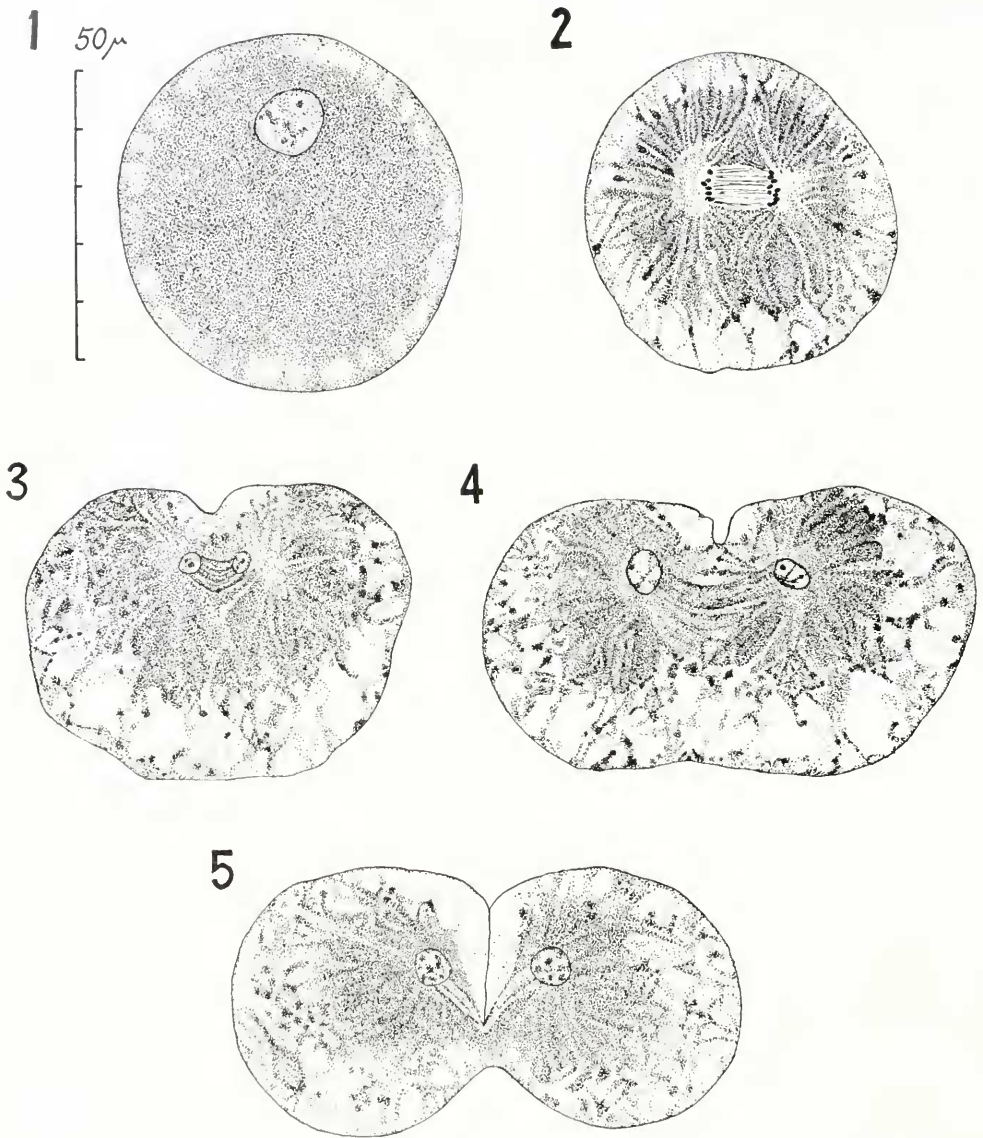


FIGURE 2. *Spirocodon* eggs fixed in chrome-formol mixture. 1, unfertilized egg. The cytoplasm is fairly homogeneous with only a slight tendency to vacuolization around the periphery. 2, telophase with chromosomes and asters. Note an intense vacuolization around the periphery. The plane of section is quasiequatorial; the peripheral vacuoles are seen encircling the island of dense cytoplasm. 3, vertical section. Anaphase with asters, a bending spindle and a shallow furrow. Notice that the island of dense cytoplasm is definitely shifted toward the animal pole. 4, a still later stage. 5, a much later stage with a V-shaped spindle. Note that the vegetal vacuoles have now come around to the former animal side.

tion of the spindle, the shape of cross-sections parallel to the cleavage plane is always circular. Similar cross-sections of medusan eggs are a series of oblongs (Plate I, *m, n, o*), and only in later stages do the flattened incipient blastomeres gradually round themselves up (Plate I, *o* and *p*). This flattening is an important point to keep in mind for an understanding of the division mechanics of coelenterate eggs.

Several other things are notable in the side view of the egg. For example, in every case observed, a vortex configuration can be seen in each blastomere during most of the cleavage process (Plate II, *B, C, D, E*). The direction of the whorl of this vortex is exactly the same as that exhibited by the astral rays of *Astriclypeus* eggs (Dan and Dan, 1947; Fig. 7), only the degree of bending of the component lines is much more acute in the *Spirocodon* egg.

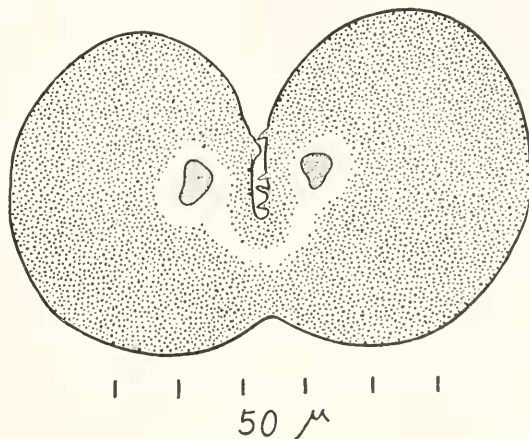


FIGURE 3. *Spirocodon* egg fixed in Flemming's solution showing homogeneous cytoplasm, V-shaped spindle and spinning processes on the furrow sides.

The next fact concerns an utterly new feature of cell activity which finds no similar case among echinoderm eggs. This is the so-called "spinning activity" of the medusan eggs.² It has been known for some time that in medusan eggs, after the cleavage furrow advances some distance, fine protoplasmic processes are sent out from the furrow sides to amalgamate into a sheet between the two blastomeres (Yatsu, 1912; Plate II).

As material for observing this spinning activity, *Spirocodon saltatrix* is not particularly favorable, for the processes are so fine that, before amalgamation, they are barely recognizable individually with an oil immersion lens (Plate II, *C'*). But the sheet, once it begins to be formed (Plate II, *D, E*), can be seen with the usual high dry objective (Plate I, *g, h*). Moreover, the partly amalgamated processes can be fixed as such by osmium (Fig. 3). Unfortunately, because of the poor visibility of *Spirocodon* processes, the details of the change from processes to sheet cannot be

² Andrews (1897) and Moore (1930) discuss spinning activity in connection with echinoderm eggs. Their views will be criticized in a future paper.

ascertained. All one can say is that the transformation is rather abrupt and as the sheet is completed, it spreads between the opposed surfaces of the blastomeres like a wetting fluid, sometimes even showing a meniscus at the optical section of its border. As the immediate result, the two blastomeres are pulled closer to each other.

The last point concerns the final positions of the divided nuclei. In either unfertilized or fertilized eggs, as long as a nucleus is visible in the living condition, it takes an extremely excentric position toward the animal pole. After it is lost from view and the mitotic spindle develops, sections show that even though the spindle has come back to a more central position, it is still decidedly pushed to the animal pole (compare Plate Ia and Fig. 2, 3 and 4). Nevertheless, when the two resting nuclei are reconstituted after cleavage their positions are about midway between the animal and vegetal poles, almost in contact with the furrow sides. This situation is quite different from that existing in *Astriclypeus* eggs, where the nuclei retain the same degree of excentricity until the four-cell stage.

Now let us see how the facts described above can be correlated with the general theory of cleavage developed in the previous papers.

ROTATION OF THE ASTERS

The direction of the whorl of the vortex configuration in the dividing blastomeres of *Spirocodon* has been shown to be the same as that exhibited by the astral rays of *Astriclypeus* eggs. In the latter case the vortex figure of the astral rays was taken as a direct indication that the asters are turning around their centers (Dan and Dan, 1947). Examination under oil immersion leaves no doubt that the figure in the *Spirocodon* eggs is also primarily due to the bending of the astral rays. Consequently, it is safe to say that the vortices in the two forms are identical in significance. Judging from Yatsu's figure (1912; Plate II, Fig. 19), similar vortices are also evident in the eggs of *Beroë forskalii*.

BENDING OF THE SPINDLE

In the previous paper, the primary cause of spindle bending in *Astriclypeus* eggs was attributed to the excentric position of the mitotic figure. It is easy to show that a similar situation obtains in the eggs of *Spirocodon*. In the unfertilized condition, the egg pronucleus is clearly visible in the living cell and it lies immediately beneath the cell membrane at the animal pole (Plate Ia). After fertilization, although sections show that the first mitotic figure comes back toward the interior to some extent, still it is clearly excentric. As cleavage proceeds, sections also reveal that the spindle is bending. This is true both for eggs fixed with chrome formol (Fig. 2) and for those fixed in osmium (Fig. 3).

Now one is able to understand why the *Spirocodon* eggs flatten during segmentation. In regularly dividing eggs where the spindle simply elongates, the force acts in linear fashion and the cell body can only elongate along the spindle axis. But in the cleavage of medusan eggs, in addition to the force which pushes the two asters apart, there is another force trying to make them turn around their excentric centers. This set of forces determines a plane on which the eggs tend to spread and make flat discs. This fact is most clearly brought out by making contour drawings

of the side view of the same egg at various stages and superimposing them. As is shown in Figure 4, the outline of the initial stage can be included within that of the flat stage.³ When the blastomeres round up again in later stages, the contour becomes smaller once more.

In passing, it must be made clear that the above statement that a set of two forces is at work in the medusan cleavage should not be taken to mean that two different factors are involved in the process. As was discussed in the previous paper (Dan and Dan, 1947), the force causing the rotation of the asters is derived from the elongating force of the spindle as a result of the excentricity of the mitotic figure. Therefore, they are nothing but two fractions of force of a single origin.

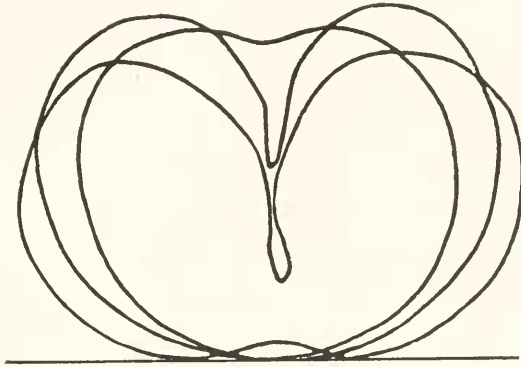


FIGURE 4. Superposition of the contours of the side-view of the same egg in three different stages. The contour of the second stage surpasses that of the first stage both in height and width, indicating that the cell body is spreading flat in this stage. In the third stage, although the width increases, the height decreases, showing that the blastomeres are rounding up.

Returning to the main line of thought, if it be correct that the flattening of the blastomeres and the bending of the spindle are two effects of a single cause, then we should have noticed the same thing in the eggs of *Astriclypeus*. Re-examination of that material shows it to be actually present, as is illustrated in Figure 5. As a matter of fact the bulging out of the vegetal contour was often noticed during that study and its probable cause was attributed partly to spindle bending, partly to astral rotation. The situation would have been grasped more readily if it had been considered in terms of flattening.

SPINNING ACTIVITY AND ITS SIGNIFICANCE WITH RESECT TO CLEAVAGE

Once it is established that astral rotation is one of the important factors in medusan cleavage, one is led to suspect that spinning activity may be closely related as a part of the division mechanism, for when the two asters are simultaneously moving away from each other and rotating around their centers, a tying together of

³ Yatsu's paper includes a somewhat similar figure of the superposition of outlines of successive stages in the cleavage of the eggs of *Beroë*. In connection with this figure, Yatsu is pointing out the absence of lateral elongation in *Beroë* eggs. But it should not be overlooked that the stages he is dealing with are those of the latter half of the division process, and the contour of the early period is not included.

the animal side of the blastomeres would greatly increase the efficacious force available for the tearing open of the vegetal furrow.

The most direct and decisive way to test this possibility is to interfere with the spinning activity and see whether or not the eggs can divide. A method of mechanical intervention was adopted. It consisted in moving a micro-dissection needle back and forth along the furrow as the latter was being formed. It was thought that this method would prevent the amalgamation of the protoplasmic processes. Special care was taken not to injure the sides of the furrow by the needle during the operation. The result was clear-cut. The eggs divided completely. But on completing cleavage, the blastomeres fell apart. Later it was found that a simpler way to achieve this aim is to make the eggs divide under compression in a hanging drop with an extremely small quantity of sea water. The tracings of photographs shown in Figure 6 illustrate an egg so treated. The reason for the failure of the spinning under compression is probably that, as the egg is flattened so much, the

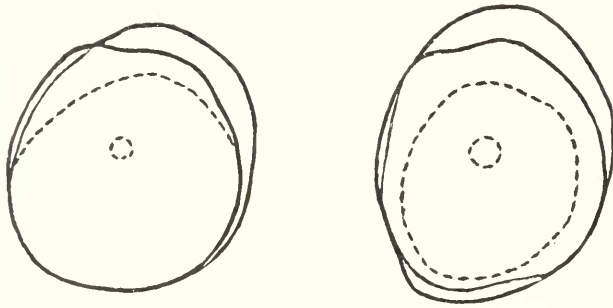


FIGURE 5. Two camera lucida drawings of dividing *Astriclypeus* eggs as seen along the spindle axis. In the left hand one, the furrow is formed only at the animal pole while in the right hand one, the furrow has gone around the cell. The large dotted circles are optical sections of the still uncleaved parts and the smaller dotted circles are cross-sections of the spindle.

number of the protoplasmic processes which are formed just at the flattened edges of the furrow sides is greatly reduced so that the chance of their meeting and consequently of their amalgamation becomes very slight.

At any rate, it can now be said definitely that the sole object of the spinning activity is to tie the blastomeres together and it can be segregated from the process of cell division proper.

PERFORATION EXPERIMENTS

In order to get some insight into the more dynamic phase of medusan cleavage, perforation experiments were made. The method is to take an egg compressed in a hanging drop and punch it through by a micropipette (Dan, 1943a).

The results obtained in sea-urchins by this method can be summarized briefly. When a perforation is made through the cytoplasm on the median plane bisecting the karyokinetic figure, the inner border of the perforation is pulled in and becomes a cleavage furrow. If a hole is shifted slightly to the side of the median plane, but still within the crossing range of the median rays (sub-median region), a furrow

is formed at the normal position, but simultaneously the perforation is drawn toward the median plane and the two are ultimately united, so that in the end a nearly-normal two-cell stage with a forked furrow results. If the hole is further pushed toward the polar region, it can no longer take part in the furrow formation.

When *Spirocodon* eggs are compressed for punching, because of the excentric position of the mitotic apparatus it is desirable to have the egg axis coincident with the plane of flattening. But unfortunately, even after compressing, the mitotic figure cannot be located definitely because of its poor visibility. Theoretically, the apparent position of the mitotic apparatus under compression can be the most excentric when the egg axis is lying parallel to the plane of flattening and it becomes

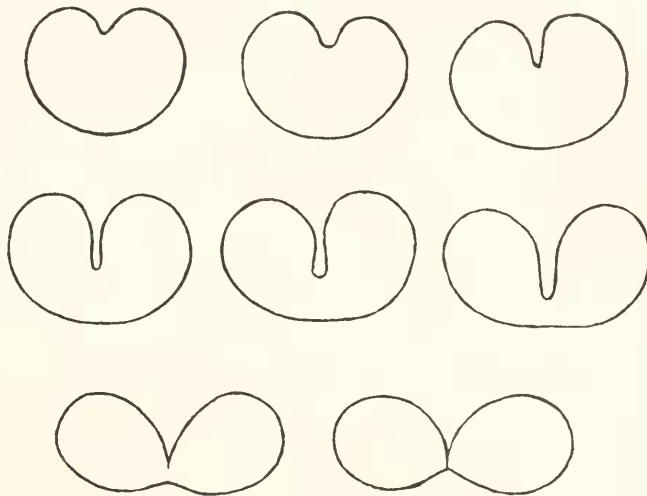


FIGURE 6. Tracings of a series of photographs of a single egg dividing under compression. Spinning has failed and the blastomeres are falling apart. In later stages which are omitted here, they are completely separated. Note, however, that until the cleavage is nearly completed (down to stage 5), the contour change of the blastomere is quite normal, indicating that these shapes are brought about by purely internal causes.

central when the egg axis is perpendicular to the plane. Another source of ambiguity lies in the fact that when a micropipette punches through the cell in the vicinity of the mitotic apparatus, there is no way of telling to which side of the pipette the mitotic figure will slip away. The final position of the mitotic figure after such treatment in each particular case can be judged to some extent by observing the way in which the furrow appears, but sometimes the authors had to guess it from the later behavior of the perforation.

The series of sketches shown in Figure 7 represents the behavior of a perforation on the median plane of the animal side. Just like the corresponding case in sea-urchin eggs, the inner border of the perforation is drawn in, becoming a cleavage furrow, while the median periphery of the cell which would become a furrow under normal conditions remains unchanged. The rest of the division process proceeds in the usual manner. At the fourth stage of the figure, a few partly amalgamated processes

are seen and, at the sixth stage, the amalgamation is complete. The division is achieved at the eighth stage. Soon after this, the mitotic figures for the second cleavage are formed and the egg cleaves into three blastomeres. This is instructive in confirming the observation that the first cleavage has failed to divide the cell completely.

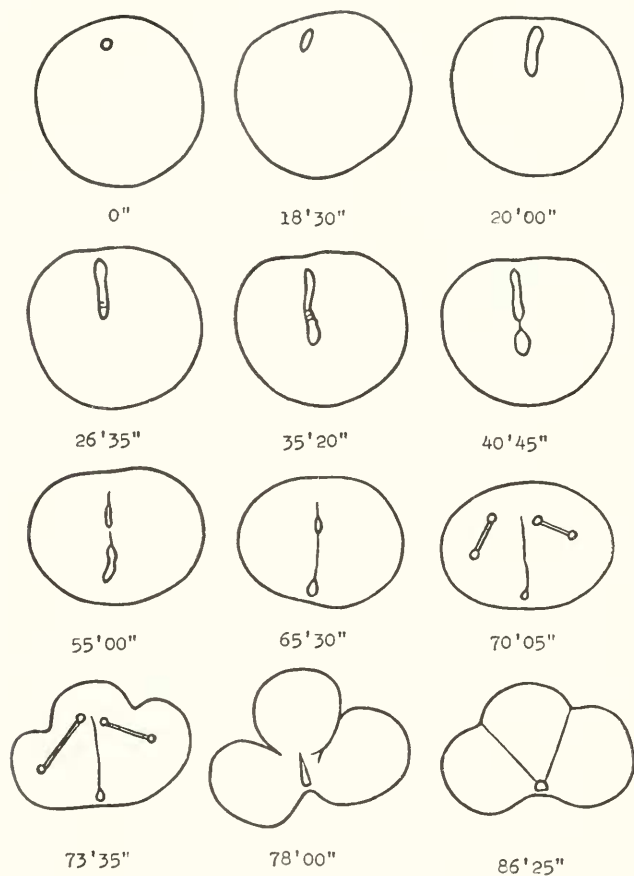


FIGURE 7. Form change of a perforation made on the animal side of the spindle. Note that it makes a cleavage furrow which takes the place of the cell periphery. From the 4th stage on, spinning activity can be seen which succeeds in binding the cells at the 6th stage. At the second cleavage, the larva reaches a 3-cell stage, the middle cell being binucleate and the other two, mononucleate. Temperature, 14.2°C .

The case shown in the next figure (Figure 8) is another example of the perforation of the animal side. In this case, although the result is identical with that of the previous example, as far as the behavior of the perforation is concerned, it is significant in showing a case of the failure of spinning. In this case, since the animal sides of the blastomeres are connected by a protoplasmic bridge, the failure in spinning causes a widening of the tip region of the furrow. As a result, a thread

of cytoplasm running between the blastomeres at the vegetal pole is clearly visible. This string of cytoplasm must be homologous to the connecting stalk of sea-urchin eggs and hence it must contain the spindle, or, more correctly speaking, the spindle remnant, within it. In the present case, however, the apparent elongation of the

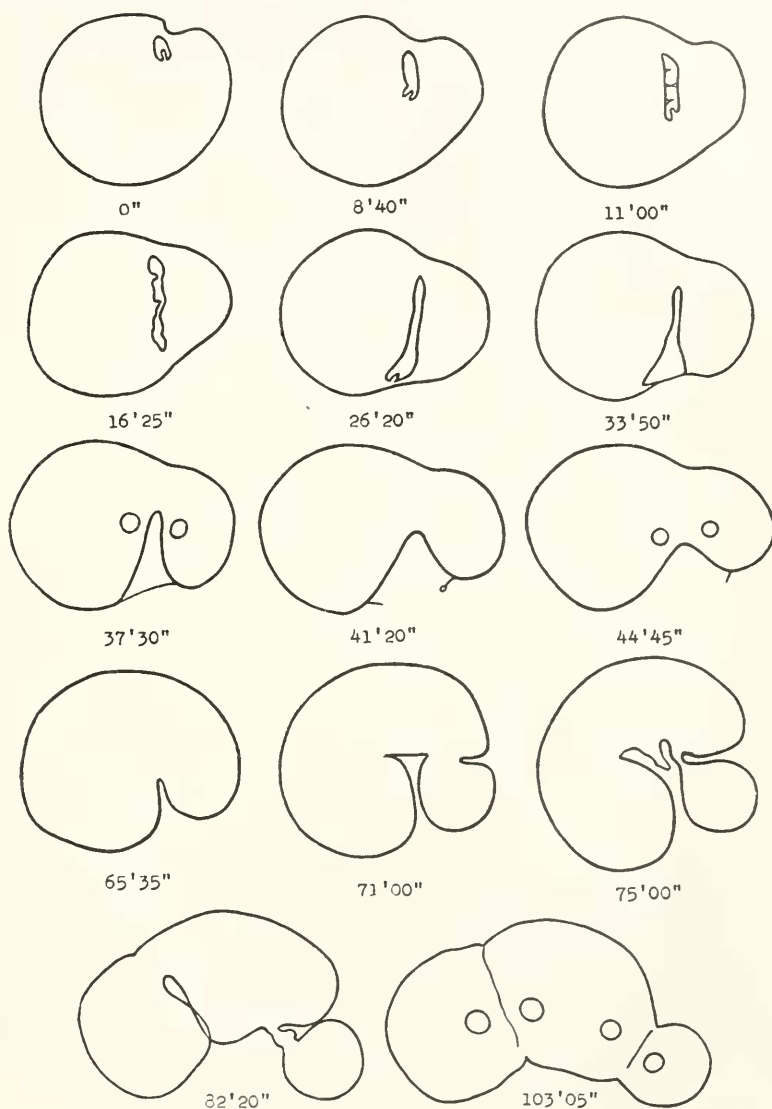


FIGURE 8. Another example of a perforation in the egg at the animal side of the spindle. At the 3rd stage threads are formed which, however, fail to bind the blastomeres. At the 6th stage, the division process is complete. The protoplasmic string left at the vegetal pole is the "stalk" and contains the spindle. At the 8th stage, the stalk is broken. At the 10th stage, the second cleavage sets in, resulting in a 3-cell stage. Temperature, 10.5° C.

spindle remnant cannot be considered to be an autonomous one but is likely a passive one due to the rounding up of the blastomeres. In the second cleavage, the egg divided into three cells, as in the previous case.

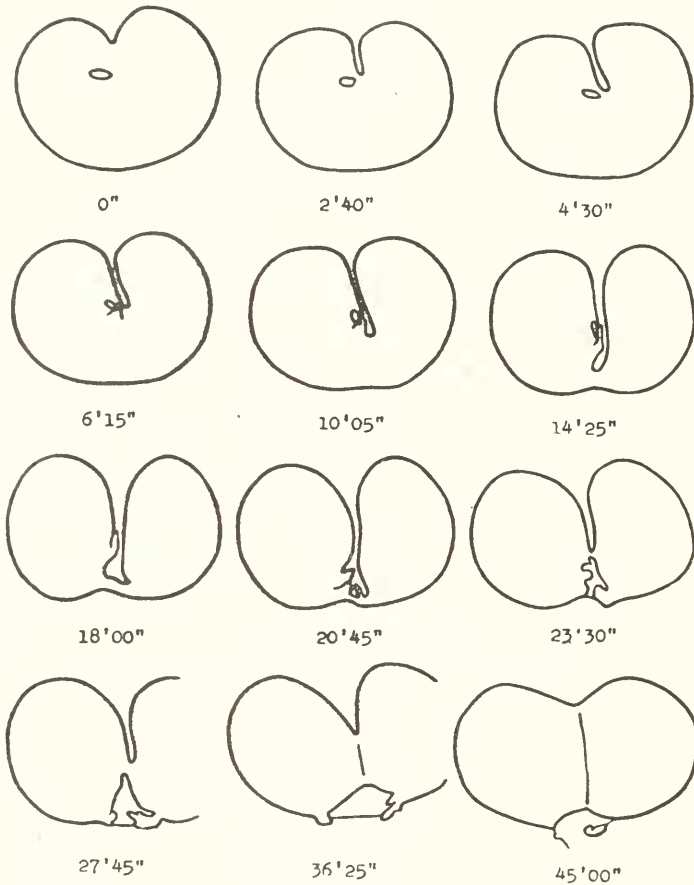


FIGURE 9. The behavior of a sub-median hole on the animal side. Immediately after the operation, the hole was round in shape, but is stretched toward the median plane by the time the 1st drawing was made. At the 4th stage, a protoplasmic protuberance appears in front of the holes. At the 6th stage, the hole is practically fused with the main furrow. At the 7th stage, the hole is stretched, practically becoming a slit so that its fusion with the main furrow cannot be ascertained. But its fusion is evident at the 8th stage through the appearance of a dent on the side of the furrow just above the protuberance. This corresponds to the Y-shaped furrow of *Strongylocentrotus* eggs. At the 9th stage, spinning is achieved at a little higher level than the dent. The spindle remnant is clearly seen at the following 2 stages and is finally broken at the 12th stage as the interkinetic condition is reached. Temperature, 13.3° C.

A typical sub-median case is given in Figure 9. In spite of some irregularities in the contour of the furrow wall, the fusion of the hole with the furrow is evident. The only superficial difference in the sub-median behaviors of the medusan and sea-urchin eggs is probably due to the difference in consistency of the cortex of the two forms.

If a perforation is pushed a little farther from the median plane to the sub-polar region, the hole is only stretched and does not fuse with the main furrow (Fig. 10). If a hole is made in the polar region, it remains quiescent all through the cleavage process (*see* Fig. 14).

In short, as far as the behavior of the perforations on the animal side is concerned, the results are just the same as those in sea-urchin eggs. But once we go to the vegetal side, the reaction changes greatly.

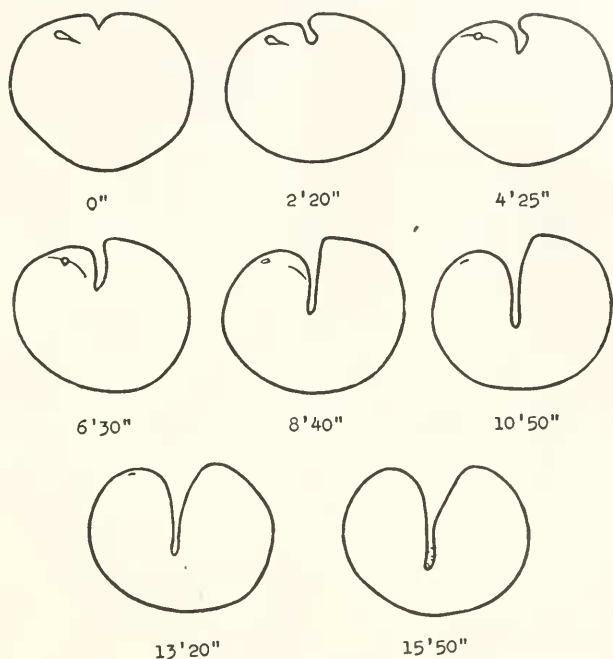


FIGURE 10. A sub-polar hole on the animal side. The hole is pulled toward the median plane. At the 3rd and 4th stages, surface grooves are formed along the direction of strain on both sides of the hole. The hole becomes smaller and slit-like at the 6th and 7th stages and finally disappears at the 8th stage.

The behavior of a median hole on the vegetal side of the spindle is shown in Figure 11. In this case, although the hole is deformed to some extent when the tip of the furrow approaches, there is no sign of the active participation of the vegetal hole in the furrow formation. The situation will more appropriately be described by saying that the furrow advances until it meets a vegetal hole where it simply stops. Therefore, the string of cytoplasm which is found on the animal side in this case must be the spindle remnant.

Here the question arises, whether the behavior of the vegetal hole approaches that of an animal hole if its position is shifted toward the upper pole. Such a case is given in Figure 12. The result is the same as in the preceding case. At the eighth stage of this figure, two nuclei have begun to be reconstructed, but the part of the spindle remnant within the cytoplasm can be recognized as bright streaks

which are continuous with the string of cytoplasm outside the cell. The shallow cleavage furrow thus formed is soon abolished completely and a binucleated cell results. At the second cleavage, the cell showed two mitotic figures. In this particular case, the cleavage attempt is unsuccessful in one blastomere and a 3-cell stage is reached at the end.

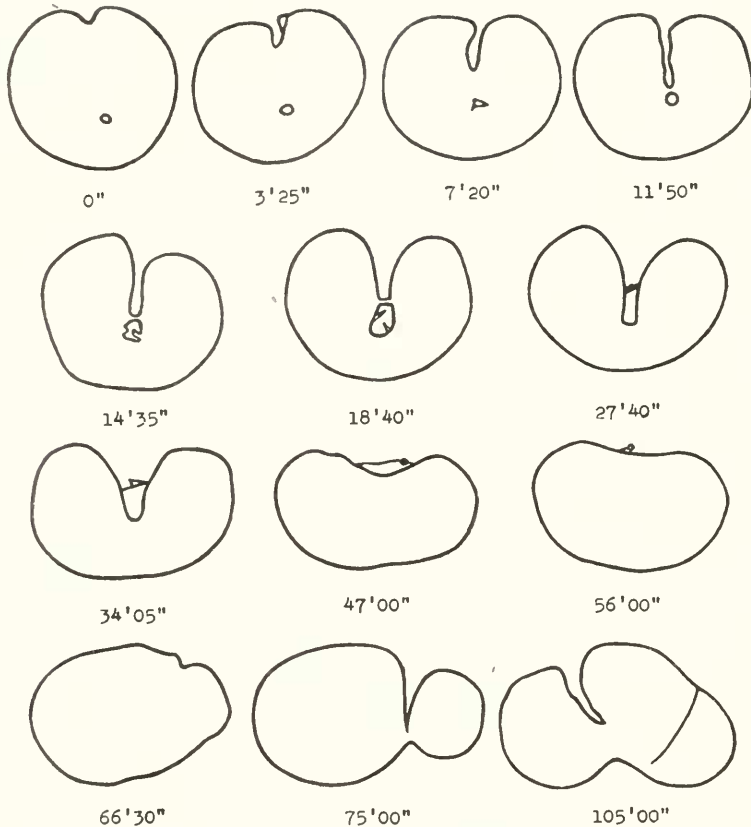


FIGURE 11. A median perforation on the vegetal side. At the 4th stage, the furrow has advanced so that only the stalk containing the spindle remains between it and the hole. At the 8th stage, the stalk is getting soft. The cell is rounding up at the 9th and 10th stages. The cell divides into three at the 13th stage. Temperature, 12.1° C.

Equally striking is the fact that as soon as a vegetal hole is shifted in position from the median plane, it no longer shows any trace of form change and often vanishes leaving only a scar on the cell surface (Fig. 13). In this egg, the hole is obliterated at the third stage, after which the surface scar left by the hole is represented by a dotted line. The instability of the vegetal hole may indicate the fluid nature of this region because perforations in sea-urchin eggs also become unstable when put in a calcium-free medium.

Combination of two holes on the vegetal side of the spindle does not introduce any new type of behavior. In the case shown in Figure 14, one hole is median while

the other hole is polar. The furrow comes down as far as the former and stops there, while the polar hole remains indifferent all through the process. In the case shown in Figure 15, the two holes come pretty close to the furrow, yet they slide along beside it and disappear later.

Summarizing the above results, it can be said that on the animal side of the mitotic figure of the medusan egg, the condition is practically the same as that pre-

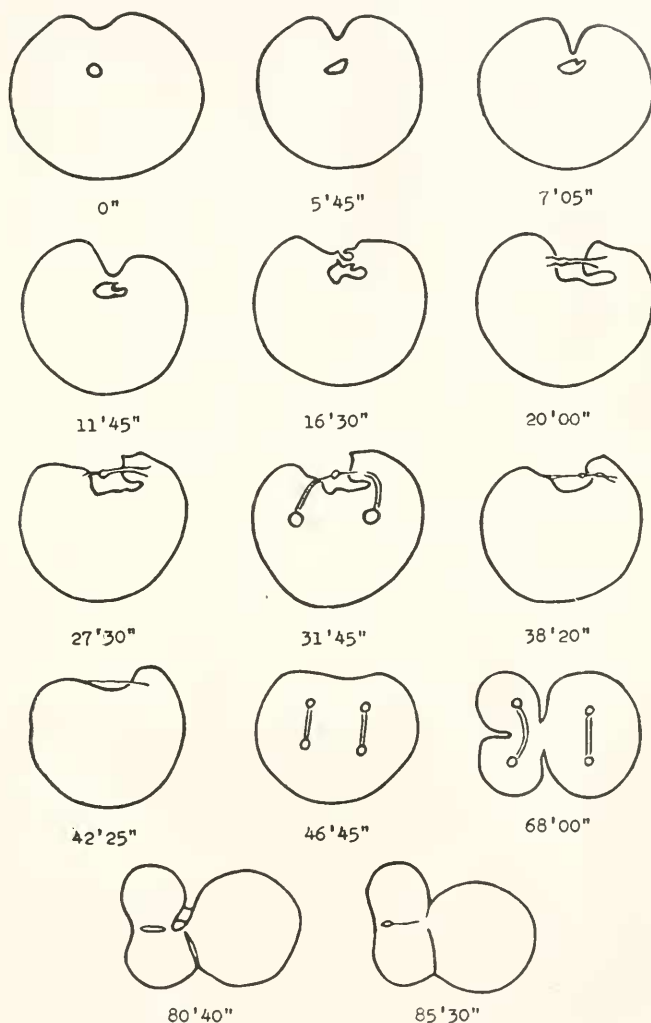


FIGURE 12. A median hole made just below the spindle. The behavior is essentially the same as in the case shown in Figure 11. The 8th stage shows that the string of cytoplasm really contains the spindle. At the 11th stage, two mitotic figures appear. In the right blastomere, the cleavage somehow fails and a 3-cell stage results. The last drawing is significant in showing that the spinning is possible not only between daughter cells but also between adjacent cells which have no common mitotic figure. Temperature, 14.2° C.

vailing in sea-urchin eggs. But the vegetal side is so inert and passive that the situation seems to be utterly different. The impression is unavoidable that the vegetal region is quite fluid. However, if the vegetal region of the medusan eggs is really fluid, is it possible to anticipate a successful cleavage from the proposed theory?

THE DIVISION MECHANISM OF MEDUSAN EGGS

As an introduction to this discussion, let us enumerate important features of medusan eggs which are not met with in sea-urchin eggs, and which, consequently,

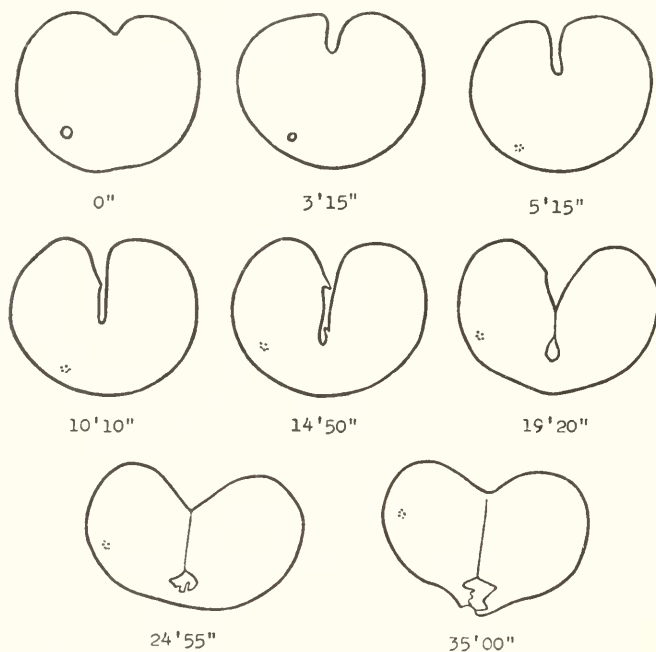


FIGURE 13. A perforation in the sub-medial region of the vegetal side of the spindle. At the 3rd stage, the hole vanishes and a surface scar remains. At the 5th stage, the spinning activity is taking place, and at the 6th stage, the two blastomeres are bound together. At the 8th stage, cleavage is complete. Note that the scar is pushed far to the side, in the polar region. Temperature, 12.1° C.

offer basic materials for the present theoretical consideration. (1) Flattening of the cell and later rounding up of the incipient blastomeres. Astral rotation has been offered as a tentative explanation of this fact. (2) Coming around of the vegetal vacuoles to the animal side in fixed eggs and the shifting in position of the surface marks left by vegetal perforations to the apparent polar region after cleavage (*see* texts of Figs. 13, 15). These two facts seem to have a close bearing on the rotation of the asters. (3) Fluid nature of the vegetal region. The instability of the vegetal perforations and the failure of the vegetal rays to exert stress on the perforations are two basic facts pointing to this conclusion. (4) Movement of the nucleus from the animal side toward the vegetal side during cleavage.

With the above four points in mind, the authors have arrived at the following conclusion. In the beginning of cleavage, the spindle begins to elongate as in the case of sea-urchin eggs. Since the astral rays of the animal region of medusan eggs are fully effective, this causes first shrinkage and later furrow formation there just as in sea-urchin eggs. As the furrow gets deeper, the median crossing rays are pushed against the spindle (sheath rays) which causes the asters to rotate. This, in turn, makes the cell spread on a plane determined by these forces of elongation and rotation. When the spindle definitely takes a U-shape, the astral centers must be twisted around through nearly 90° from their former directions.

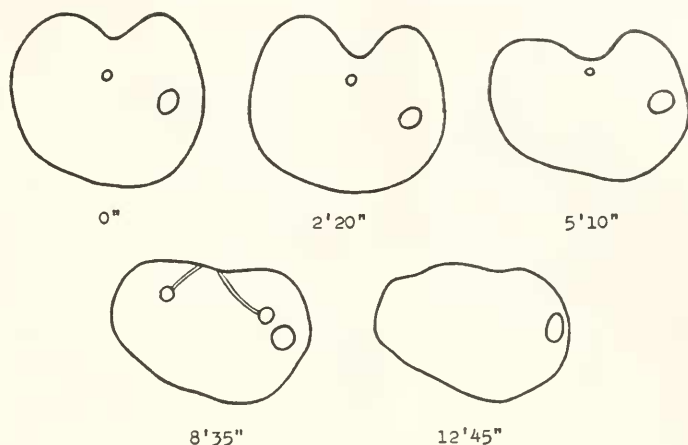


FIGURE 14. Combination of a median and a polar hole. Notice that the polar hole is not affected at all. The behavior of the median vegetal hole remains unchanged by the presence of the polar hole.

Now, if the astral rays of the vegetal region were rigid enough, this condition would immediately cause a fission in the vegetal region between the two asters as in the case of the *Astriclypeus* eggs. But, actually, the vegetal portion of *Spirocodon* eggs is much more fluid and more deformable. Therefore, instead of tearing the vegetal cortex apart (as in typical furrow formation) the cortex and the rays are both stretched out, allowing the central parts of the asters to rotate. In other words, in *Astriclypeus*, the annulment of the strain exerted by the elongating spindle is achieved by the breaking of the cortical layer (furrow formation) while in *Spirocodon* it is accomplished by the yielding of the cortex and the rays to the strain (without furrow formation).

What the authors think about the condition of the rays and the cortex is shown diagrammatically in Figure 16. The cleavage stage corresponds to stage B of Plate II. The thin lines represent extremely stretched parts of the rays and of the cortex while the thick ones are the parts which are stretched slightly or not at all. According to our interpretation, there are no rays which go to the furrow side. This ray-free narrow strip lining the furrow side must correspond to the "fan-shaped ray-free area" described in connection with *Astriclypeus* eggs (Dan and Dan, 1947). (In *Astriclypeus* eggs, as the median crossing rays are caught by the

furrow head and are pushed in as sheath rays, non-crossing rays on both sides are left behind. As a result, a fan-shaped ray-free gap appears.) In *Spirocodon*, since the arc of the fan-shaped area is stretched out so much, the shape of the fan is greatly flattened. This explains why the daughter nuclei lie so close to the furrow wall. It might be thought that data like those of Figure 16 could be obtained directly by the kaolin method. However, this is impossible since no particles can stay attached to *Spirocodon* eggs.

As the division process approaches its completion and the soft vegetal region is stretched out more and more, the central portions of the asters are released from the strain and regain their former condition. This is expressed as the rounding

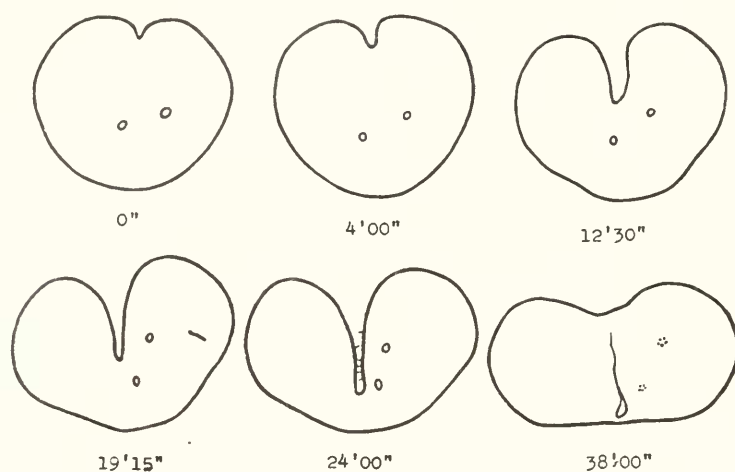


FIGURE 15. Two vegetal holes. The furrow cuts in on one side of the holes. In spite of the fact that the furrow passes quite close to the holes, no change is brought about in their shape. They vanish later. Note also that the direction of the line connecting the two holes is nearly horizontal in the beginning, becoming almost vertical after cleavage is complete. This shows that vegetal cytoplasm before cleavage comes around to the side during cleavage.

up of the incipient blastomeres in later stages of the division process. Moreover, such an astral rotation will bring about the rotation of other cell constituents. This must surely be the explanation of the movement of vacuoles and surface scars from vegetal to more animal positions.

However, the last and the most important point is that, as the vegetal material is carried around to the side and even up toward the animal pole, the relative position of the spindle within the cell will become more and more vegetal and finally the base of the U-shaped spindle will reach the vegetal extremity of the cell. In a very rough approximation, it is as if one cuts a medusan egg into two along the cleavage plane, turns the halves by 90° (so that the cut surfaces face downward) and pushes them together animal pole to animal pole. The cleavage is thus complete, with the nuclei in the vegetal region.

When looked at from a different angle, the above theoretical consideration brings forward two more propositions.

(1) It was stated that as the imposed strain is equalized, during the latter half of the cleavage process, the vegetal material is shifted around toward the animal side and the spindle is shifted down. These coupled movements are an action and a reaction. As a result, the authors cannot agree with the opinion that a cleavage furrow strives to reach the opposite pole by its own power.

Such an illusion must have arisen from the fact that when a cleaving medusan egg is observed, the only thing which can be seen moving is the cleavage furrow, and the cell body remains practically stationary. But this is due to a simple physical condition. When a cleaving medusan egg comes to lie on its side as the result of flattening, it must be resting on the substratum by two points, with the center of gravity of the cell somewhere on the line connecting these supporting points.

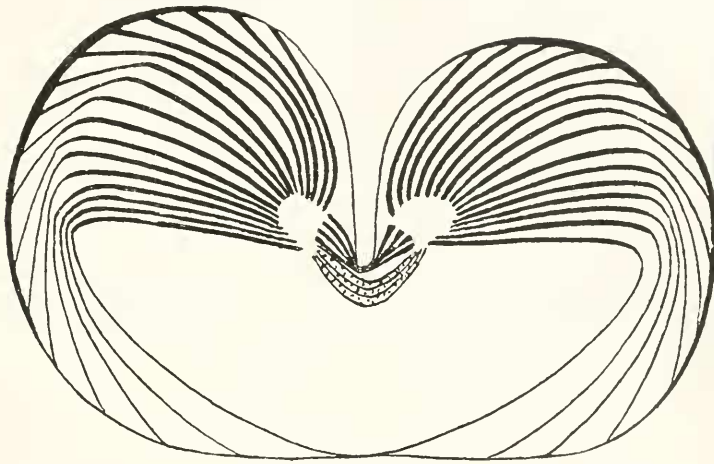


FIGURE 16. The condition of the astral rays and the cortex as interpreted by the authors from stage *B* of Plate II. The distal portions of the vegetal rays are greatly stretched. The animal cortex is drawn into the furrow and the vegetal cortex is stretched to cover the lower half of the blastomeres. Greatly stretched parts are represented by thin lines. The ray-free area is spreading along the furrow wall. This figure is only a rough qualitative representation.

Therefore, no matter what change occurs in the contour, as long as the center of gravity does not change its position, the cell cannot help remaining stationary. The rotational movement of the aster such as was discussed above will certainly not shift the position of the center of gravity very much. In other words, supporters of the opposing theory made an error in selecting the axes of coordinates. They took the more or less stationary cell contour as a reference and compared the movement of the furrow head with it. But if we have to choose a reference point, it seems to us that the position of the nuclei will be a far more reliable one. To illustrate this proposition, a series of camera lucida drawings of a dividing *Spirocodon* egg were superimposed in such a way that the nuclei would coincide as nearly as possible. The result, Figure 17, shows clearly how the vegetal material is carried up toward the animal side. If a dividing medusan egg could be suspended in some medium during cleavage, it might give the same sort of effect.

(2) The impetus for the present research was the expectation that the cause for the difference between the modes of cleavage of *Astriclypeus* and *Spirocodon* eggs was probably attributable to the difference in the degree of excentricity of the spindle. But this expectation turned out to be not strictly correct. The accurate statement must be that the difference in the mode of cleavage of the two forms lies in the difference in rigidity of the vegetal halves of the asters. Among *Astriclypeus* eggs, there is, of course, an undeniable correlation between the degree of the spindle excentricity and the degree of the onesidedness of the furrowing (Fig. 1). But this is, in turn, because there also exists a correlation between the degree of

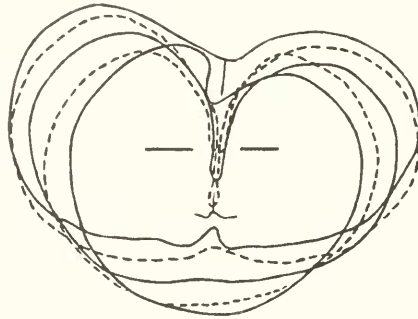


FIGURE 17. The superposition of contour drawings of a dividing *Spirocodon* egg, keeping the nuclei in as nearly a constant position as possible. But the nuclei cannot be made to overlap exactly. Their positions fall on the two lines indicated in the center. The shifting of the vegetal material to outer sides and up to the animal side is evident.

spindle excentricity and the degree of deformability of the vegetal halves of the asters.

DISCUSSION

Cytoplasmic streaming and astral rotation

Prominent among the theories which have been put forward to explain the division mechanism of medusan eggs is the view that a cytoplasmic current, considered to originate from surface tension changes, is the effective force. This theory seems to have stood partly upon the positive fact that directed movement of the cytoplasmic granules toward the tip of the furrow has been reported ever since the early days of embryology, and partly upon the negative fact that the theory has not been challenged by an alternative one. It certainly created the impression that the phenomenon of cytoplasmic streaming has special prestige in the domain of cleavage studies of medusan eggs and some investigators have even gone so far as to think that it is also true in sea-urchin eggs.

To the authors, however, there seems to exist a profound difference between the streaming in sea-urchin eggs and that in medusan eggs. The streaming in sea-urchin eggs is a current of cytoplasm toward the future cleavage plane, hence it is a current flowing prior to furrow formation (Chambers, 1938; Just, 1939, p. 284). On the contrary, the reports on medusan eggs are all concerned with cytoplasmic currents along the already formed cleavage furrow converging toward the furrow

head. Therefore, even allowing for the possibility that streaming is a chief cause of cell division, the modes of action of the two streamings must be entirely different.

Moreover, the literature on medusan cleavage is further complicated by the existence of ectoplasmic movement. As is known, the ectoplasm of *Beroë* eggs changes its thickness and usually thickens around the cleavage head (Spek, 1926). Since the ectoplasm accumulation occurs at the point toward which the cytoplasmic currents are directed, the former is often thought of as the result of the latter. But these two ought to be clearly differentiated. In short, within the vague term of "cytoplasmic streaming," three different things are included: namely, sea-urchin egg streaming, medusan egg streaming and the medusan ectoplasmic movement.

As for the streaming of sea-urchin eggs, the senior author has offered the interpretation that it is a passive movement of the fluid cytoplasm to fill in the gap formed between the separating asters. Concerning the streaming of medusan eggs, the authors are still more skeptical. They think that this streaming itself might be an illusion due to the *en masse* rotation of the asters. It must be remembered that when other investigators talk about a cytoplasmic current, what they are actually seeing is a movement of suspended granules. It is proposed that the mere fact that the granules move in reference to some landmarks outside the egg cannot have finality in the conclusion that the cytoplasm is flowing. The conclusion is allowable only when the granules move in reference to other cell structures such as the cell membrane. In the case of sea-urchin eggs, such a guarantee is provided because the streaming, after having reached the site of the future cleavage plane, is said to change its course and go inward. Since no such guarantee is available in the case of medusan eggs, who can say that it is only the cytoplasm which is moving? Furthermore, if it is a real streaming, why is there not found an accumulation of granules beneath the furrow head where the ectoplasm is thick?

According to Spek (1926), the cytoplasmic currents of *Beroë ovata* flow along the furrow sides, meet at the furrow tip and go a short distance to the inside along the median plane. This fact, he contends, is an evidence that the higher surface tension at the furrow head is causing the one-sided furrow formation. But to our surprise, on reading his paper carefully, we do not come across any streaming in the strict sense. The arrows indicating the direction of the flow in his text figures are only representing the directions of an hypothetical streaming if such a streaming could be considered to be the cause of the movement of the green ectoplasm. On page 60, he writes, "Während dieser Substanz Verlagerungen konnten Plasmaströmungen direkt nicht wahrgenommen werden. Konstruiert man aus dem Effekt der Plasmaverlagerungen das Strömungsbild, so erhalten wir ein eindeutiger Weise einen oberflächlichen Zustrom nach der Furche und einen axialen Abstrom von derselben Weg (S. Textabb. 3)." In other words, this is only a "Strömungsbild" where no streaming is seen. But from the authors' standpoint, the fact that his arrows are indicating the exact directions of the rotation of the asters is too significant to be overlooked.

Ectoplasmic movement and aster rotation

As for the movement of the ectoplasmic layer of *Beroë ovata*, the authors are of the opinion that this is again a passive movement. The ectoplasmic thickening at the beginning of furrow formation must be the result of the

shrinkage of the furrow surface at this stage as has been observed so many times in the kaolin experiments of sea-urchin eggs. The accumulation of the green ectoplasmic material at the points of the last connection between the blastomeres can be accounted for as follows. When the vegetal pole is stretched out and is about to be torn, the connecting bridge between the blastomeres must almost exclusively consist of the cortical material without including fluid endoplasm. Therefore, when it finally gives way, there ought to be an accumulation of the green material. During the intermediate stages, the surface area of the furrow region shrinks slightly (from unpublished data on sea-urchin eggs). Consequently, the ectoplasm around the furrow head can retain the thickness illustrated in Spek's figures.

But far more important is the later behavior of the green ectoplasm. According to Spek, in the first two cleavages, the green material which accumulates around the point of the last connection once more distributes itself evenly around the blastomeres. But at the end of the third cleavage, its re-distribution is prevented and even the nuclei are held there. As a result, at the fourth cleavage, the furrow starts to be formed at the opposite pole of the larva (the micromere pole). But this cleavage and the following one (the fifth) being extremely unequal, two sets of micromeres consisting entirely of the green material are formed. These micromeres are the mother cells of the future combs. The macromeres above them are still retaining the residue of the green material adjacent to the micromeres. In other words, as long as the cleavage is extremely unequal, the green substance stays on the same side of the larva.

After a long resting period extending for nearly two hours, the macromeres go into a division. This division is equal and the furrow starts to cut in from the micromere pole. As the furrow advances toward the other side (the polar-body pole), the last trace of the green material is carried away with it and at the completion of the cleavage, the green substance is found at the other (polar body) pole. In the following extremely unequal cleavage all the green material is given off as micromeres. As is clear, this new set of micromeres lies opposite from the group of comb-forming micromeres and they are endodermal elements.

From the authors' concept of medusan cleavage, this back and forth movement of the green ectoplasm is not surprising, since medusan blastomeres are turning somersaults at each division. When the division is unequal, although the same turning around accompanies it, the division cannot take the ectoplasmic material across the large cell and apparently leaves it in place. So it must always be an equal division which can transfer the material across to the opposite side.

In the field of amphibian embryology, the opinion is often expressed that the process of amphibian gastrulation is a formative movement by which various mesodermal elements are delivered to their definitive positions. In that sense, it is exceedingly interesting that the cleavage process itself is acting as a formative movement in *Beroë*.

The reason why the green ectoplasm re-distributes itself evenly after the first two cleavages is unknown to the authors. Spek thinks that it is due to the low plasma-viscosity during these mitoses. Whatever the explanation is, the phenomenon of the complete re-distribution of the ectoplasm seems to have a close bearing on the fact that the cleavage furrows cut in from the animal pole (the polar-body

pole) during this period. In *Spirocodon saltatrix*, on the other hand, the redistribution is prevented even for the first cleavage. As a result, the nuclei come down half way between the two poles, lying quite close to the furrow surface, and the cleavage furrow of the second division starts from there. This is functional evidence that the animal pole has come around to the furrow side and the vegetal material is spread out to cover the lower half of the blastomeres.

Medusan embryology and aster rotation

The concept of the rotation of the blastomeres of medusan eggs seems to offer a hint with respect to a more general problem of the embryology of ctenophores. Among this group, it has been a puzzling fact that the egg axis and the larval axis run in opposite directions. This means that the apparent vegetal pole of the egg where the comb-forming micromeres appear becomes the fore-end of the swimming larva. This is quite contrary to the situation found in other animal phyla. For this reason, investigators who put emphasis on the later development name the two poles of ctenophore eggs exactly opposite to the way it is customarily done, calling the polar-body pole the vegetal pole (Schleip, 1929; Korschelt, 1936). Korschelt even goes so far as to illustrate the eggs up-side-down. All these confusions have their origin in the fact that people view the development of ctenophores with the conventional idea that the egg axis should coincide with the larval axis. But if the blastomeres turn upside down at each division, the egg axis changes its direction each time. In other words, among medusae, the egg axis seems to have little meaning.

SUMMARY

1. Characteristics of division involving an excentric mitotic figure: namely, astral rotation and spindle bending, are recognizable in the division of *Spirocodon* eggs.
2. Perforation experiments indicate that although the physical condition of the animal region of *Spirocodon* eggs is similar to that of sea-urchin eggs (*Pseudocentrotus depressus*), the vegetal region is quite fluid and deformable.
3. In *Astriclypeus*, the vegetal region is like the animal in physical consistency so that the vegetal region is pulled open (i.e., furrow formation takes place) sooner or later. In *Spirocodon*, the vegetal region is quite fluid. As a result, it is never pulled open but is simply drawn out until it finally breaks. This is the fundamental difference between heart-shaped cleavage and medusan cleavage.
4. Astral rotation is discussed in connection with cytoplasmic streaming, ectoplasmic movement and medusan embryology generally.

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